c.) Remarks

As of this response, claims 20, 22-24, 26-28, and 35 remain pending. Claim 1 has been amended to introduce the following: (1) the aromatic substrate is chlorinated, (2) oxidation is carried out in the presence of an electron transfer redoxin, (3) the enzyme is a P450 monooxygenase with specific mutations. Claim 35 has been converted to a process claim dependent on claim 20.

Outstanding Rejections

1. The Examiner has rejected claims 20-28 and 35 under 35 USC § 112, first paragraph, as containing subject matter which was not described in the specification is such a way as to reasonably convey to one skilled in the relevant are that the inventors, at the time the application was filed, had possession of the claimed invention.

2. The Examiner has rejected claims 20-28 and 35 under 35 USC § 112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention.

3. The Examiner has rejected claims 20-28 and 35 under 35 USC § 103(a) as being unpatentable over WO 96/14419 (Flitsch et al) of U.S. Patent No. 6,100,074 (Flitsch et al) and Shimohi et al (Biochemistry (1998), 37, 8848-52).

4. The Examiner has rejected claims 20-24 under the judicially created doctrine of double patenting over claim 8 of U.S. Patent No. 6,100,074.

Rejection of Claims 20-28, and 35 under 35 USC § 112, first paragraph

The claims have been limited to the use of a $P450_{cam}$ enzyme that comprises specific defined mutations or a P450 monooxygenase with the equivalent mutations. As described in the Examples of the application of the inventors have shown that substitution of amino acids

in the active site of $P450_{cam}$ to less polar amino acids increases the ability of this P450 enzyme to oxidise ring carbons of halogenated aromatic substrates. The structure to function relationship of the enzymes of the P450 family of monooxygenases is known in the art. As can be seen from the enclosed copy of Gotoh et al (1992) J. Biol. Chem 267, 83-90 methods of accurately aligning the sequences of P450 enzymes have been developed. Further as discussed on page 88 right hand column lines 3 to 5 such alignments are in accordance with experimental data which identifies substrate recognition sites in P450 enzymes. In other words the skilled person would be able to use such alignment methods to determine the equivalent functional regions or equivalent amino acids of another P450 enzyme, in particular in combination with the algorithms mentioned on page 6 lines 13 to 21.

Gotoh et al identifies the six regions of P450 enzymes which acct as common substrate recognition sites, and therefore demonstrates that not only do these enzymes have similar sequences, but they also share the same structure/activity relationships, i.e. sequences which are similar between different P450 enzymes have similar functions in the different enzymes. Thus the teachings of the present work which has been carried out on $P450_{cam}$ are applicable to any P450 enzyme since the mutations mentioned in claim 1 would be expected to cause increased activity towards halogenated aromatic compounds in all P450 enzymes.

Claim 1 has been amended to specify that the substrate is a chlorinated aromatic compound which has more than one chlorine atom. Unlike classical enzyme catalysis the activity of P450 enzymes does not depend on transition state stabilisation, but instead on the ability of a substrate to access the haem group of the enzyme. Thus whether or not a substrate will be oxidised is dependent on whether the relevant amino acids of the P450 enzyme allow the substrate access to the haem. This means that the actual structure of the

substrate is much less important, and therefore the mutant enzymes mentioned in claim 1 would be expected to have increased activity towards any polychlorinated aromatic substrate.

This is supported by pages 88 to 91 of the enclosed Mueller et al article show how many mutations which affect P450 enzyme activity are mutations which affect substrate access to the haem. Therefore in mutant P450 enzymes which have different activities the reason for the difference in activity is often access to the haem, and not the inability to form a transition state structure. Given this P450 enzymes will oxidise a broad range of substrates.

Further, the Examples of the application show oxidation of a range of polychlorinated benzene and biphenyl compounds by mutant P450 enzymes demonstrating that P450 enzymes are able to oxidise substrates which have very different structures and sizes.

The Examiner also comments that the application does not contain adequate teaching about how a contaminated locus may be treated using mutant P450 enzymes (the subject of claim 35). Claim 35 has been amended so that it is dependent on claim 20. The skilled person would know how to use a mutant P450 enzyme of the invention to treat a contaminated locus, for example by applying a bacterium which expressed the enzyme. Formulations of the enzyme which can be used to treat the locus are known and therefore the skilled person could carry out the process of claim 35 without any additional teaching being required. The exact manner in which the mutant enzyme is applied is not related to the inventive concept of the claimed matter, and therefore further description of how the enzyme is to be applied to a locus is not required in the application.

Therefore, once the skilled person had been provided with the information in the present application it would be a routine matter for them to treat a contaminated locus, taking into account relevant factors such as the nature of the site and the extent of the contamination.

The applicants also wish to stress that the claims are limited to the use of the mutant P450 enzymes to oxidise polychlorinated aromatic compounds, and do not claim the mutant enzymes *per se*. Oxidation of polychlorinated aromatic compounds by P450 enzymes with the specific mutations of claim 1 is demonstrated in the Examples of the application. Therefore the claims fairly reflect the contribution which the applicants have made to the art, and are not unduly broad.

Thus the invention is sufficiently described in the application, and the applicants were in possession of the invention at the filing date.

The Examiner notes that the positions of the mutations in the claims are not defined with reference to a SEQ ID NO. The description defines the amino acid positions of $P450_{cam}$ using the standard nomenclature in the art. The same nomenclature is also used, for example in Flitsch et al (WO 96/14419) which has been cited by the Examiner. Therefore the skilled person reading the application would be in no doubt as to the positions of the mutations specified in claim 1, and amendment of the claims to refer to a SEQ ID NO is not necessary.

Rejection of Claims 20-28, and 35 under 35 USC § 112, second paragraph

The term "halo aromatic substrate" has been replaced by "chlorinated aromatic substrate" in the claims. Applicants believe that this addresses the examiner's indefiniteness rejection under 35 USC 112, second paragraph.

It is believed that the amendments to claim 35 overcome many of the objections which the Examiner raises against this claim. In particular this claim no longer refers to cells and therefore the construction of the cells or how they express the mutant P450 enzyme is no longer relevant. It must be mentioned though it would be routine matter for the skilled person to produce a cell which expresses the mutant P450 enzymes of claim 1 using recombinant DNA technology.

The Examiner objects to the term "locus" as not being unclear. However this term is clear in the context of the invention,. Page 9 line 32 to page 10 line 1 make it clear that a locus is essentially a site such as land or water that can be decontaminated using the process of the invention. Any further definition of the site to be treated or the manner in which the treatment is to be carried out is not required as this does not relate the inventive concept, and the skilled person could use routine available means to carry out treatment on any site.

Claim 20 has been amended to refer to an electron transfer reductase and an electron transfer in accordance with the Examiner's suggestion.

Rejection of Claims 20-28, and 35 under 35 USC § 103(a)

The Examiner has rejected the pending claims under 35 USC 103(a) based on Flitsch et al (WO 96/14419), Shimoji et al and Gooch et al.

The present invention provides a process for oxidising the ring carbon of a polychlorinated aromatic compound. Oxidation of such carbons is an important first step in the breakdown of these aromatic compounds. However, the oxidation of the ring carbons of polychlorinated aromatic compounds is difficult, and in particular enzymes which catalyse this oxidation are not available. The present invention is based on the finding that substituting particular amino acids of a P450 enzyme dramatically increases its activity towards a range of polychlorinated aromatic compounds. As can be seen from the table on page 23 of the application, although wild-type $P450_{cam}$ has negligible activity towards polychlorinated aromatic compounds, a $P450_{cam}$ enzyme with mutations at the positions mentioned in claim 1 has substantially increased activity towards such substrates. There is no teaching in the prior art that suggests that mutations in P450 enzymes can alter their substrate specificity so that they can oxidise a ring carbon of a polychlorinated aromatic substrate, and

therefore no motivation in the prior art for the skilled person to carry out the process of claim 1.

Turning to the documents cited by the Examiner, we note that the Examiner acknowledges that although Flitsch et al discloses mutant P450 enzymes it does not teach the oxidation of halo aromatic compounds having more than one halogen atom or suggest the use of P450 enzymes for such an oxidation.

The Examiner notes that Shimoji et al discloses the oxidation of 4-chlorotoluene to 4-chlorobenzyl alcohol. This oxidation is of course not oxidation of a ring carbon as required by claim 1, and it is important to note that oxidation of the methyl group of toluene is easier than oxidation of a ring carbon. Further this document does not disclose an enzyme which has a mutation as defined in claim 1. Thus the applicants submit there is no teaching in this document which is relevant to the present invention. The document does not provide any suggestion of using the particular mutant P450 enzymes of claim 1 to oxidise the ring carbon of a polychlorinated aromatic compound, and further contains no data that suggests that such an oxidation was possible.

Gooch et al discloses the induction of natural P450 enzymes in scup in the presence of particular aromatic compounds. However, there is no disclosure of oxidation of polychlorinated aromatic compounds by P450 enzymes. As mentioned at lines 9 to 12 of the abstract of this document on page 422 this document discloses ethoxyresorufin O-deethylase (EROD) activity, levels of immunodetectable cytochrome P450E and P450E mRNA levels (shown in Tables 1 to 4). Resorufin is 7-hydroxy-3H-phenoxazin-3-one and so is not a polychlorinated aromatic compound. Therefore Gooch et al provides no indication of whether or not P450 enzymes are able to oxidise polychlorinated compounds, and in particular has no teaching concerning the use of mutant P450 enzymes.

Thus the cited prior art documents do not provide any teaching which would lead the skilled man to believe that P450 enzymes could be used to oxidise polychlorinated aromatic compounds. In particular these documents do not suggest the use of mutant P450 enzymes to oxidise such compounds, and further provide no indication that P450 enzymes with the specific mutations mentioned in claim 1 can oxidise polychlorinated compounds. Therefore the present claims are not obvious from the cited documents.

Rejection of Claims 20-24 under Obviousness-Type Double Patenting

Because of the present amendments, base claim 20 now requires the presence of electron transfer redoxin. Because this is not present in claim 8 of U.S. Patent No. 6,100,074, applicants assert that claims 20-24 are not obvious in view of the aforementioned claim. Accordingly, applicants respectfully request that the examiner withdraw the double patenting rejection of claims 20-24.

Moreover, it is known to those of skill in the art that poly-halogenated compounds are difficult to oxidize. It would not be clear to one of skill in the art, upon reading the '074 patent, that its teachings could be used to oxidize such compounds.

d.) Conclusions

Claims 20, 22-24, 26-28, and 35 remain pending as of this response. In light of the arguments made herein, Applicants assert that the pending claims are now in condition for allowance. Because the Examiner's requirements have been satisfied, Applicants respectfully request withdrawal of the outstanding rejections Accordingly, Applicants earnestly request allowance of the pending claims. This is intended to be a complete response. If any issues remain outstanding, please contact the undersigned for resolution of the same.

Applicants enclose a check for $110.00 for a one-month extension of time associated with the filing of this document. Applicants believe that no fees are associated with the filing of this document. However, if Applicants are in error, the Commissioner is hereby authorized to draw any required fees associated with this filing, from Deposit Account No. 06-2375, under Order No. P02353US1/10112404, from which the undersigned is authorized to draw.

Respectfully submitted,

Date: May 19, 2003

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Date: May 19, 2003 By _____ (Pamela Tincha)